



## Phytochemical Investigation and Evaluation of Antibacterial Activity of Zizyphus Xylopyrus (Retz) Willd Leaf Extract

Biswaranjan Das<sup>1</sup>, Preeti Mangala<sup>2</sup>, Neha Joshi<sup>3</sup>, Rahul Subhash Buchade<sup>4</sup>, Rekha S<sup>5</sup>, Rajarshi Nath<sup>6</sup>, Abinash Kumar Sahu<sup>7</sup>, Arun Mittal<sup>8</sup>

<sup>1</sup>Centurion University of Technology and Management, Gopalpur, Balasore, Odisha, India. 756044

<sup>2</sup>Department of Chemistry, Jyoti Nivas College Autonomous, Koramangala, Hosur Road, Bangalore, Karnataka. 560 095

<sup>3</sup>Assistant Professor, College of Pharmacy, Graphic Era Hill University, Dehradun Graphic Era Hill University, Bhimtal Campus, Nainital, Uttarakhand. 263136

<sup>4</sup>Department of Pharmaceutical Chemistry, SCES's Indira College of Pharmacy "Niramay", S.No.89/2A, New Pune Mumbai Highway, Tathwade., Pune, Maharashtra, India. Orchid id: 0000-0002-8825-0922

<sup>5</sup>Department of Pharmaceutical Chemistry, College of Pharmaceutical Sciences, Dayananda Sagar University, Bangalore. 560078

<sup>6</sup>Department of pharmaceutical chemistry, Bharat Technology, Uluberia, Howrah, & JIS University, 81, Nilgunj Rd, Jagarata Pally, Deshpriya Nagar, Agarpara, Kolkata, West Bengal. 700109

<sup>7</sup>The Pharmaceutical College, Samaleswari Vihar, Tingipali, Barpali, Bargarh, Odisha.768029

<sup>8</sup>SRM Modi Nagar College of Pharmacy, SRMIST, Delhi-NCR Campus, Ghaziabad, Uttar Pradesh, India. 201204

| Article History                                                                       | Abstract                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|---------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Received: 06 September 2023<br>Revised: 05 October 2023<br>Accepted: 11 November 2023 | An experiment was carried out to evaluate the antibacterial activity of the Zizyphus xylopyrus ethanolic fractions, which was related to the phytoconstituents present. The plant's root powder was extracted using an ethanol extraction procedure that involved several consecutive steps. Following this, the extracted product displayed a distinct scent feature. Tannic acid, phenol, and flavonoids were found, indicating the existence of the required phytochemicals. The flavonoids were isolated using spectroscopic characterisation using the ethanolic extract. After then, this extract was used for additional pharmacological testing because in the study, only ethanolic extracts and saponins were used. Based on each sample's results from a variety of qualitative tests, this analysis was carried out. The discovery of new illnesses, especially those brought on by Enterococcus and Staphylococcus species, has sparked increased interest in the study of therapeutic plants in recent decades. These microorganisms have become resistant to widely used antibiotics and are the cause of a considerable proportion of hospital-acquired illnesses. For example, S. aureus, which was once sensitive to a number of antibiotics, is now showing signs of resistance to several medications. |
| CC License<br>CC-BY-NC-SA 4.0                                                         | <b>Keywords:</b> Zizyphus xylopyrus, Isolate Fraction, phytochemicals, bioactive compounds etc.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |

## **1. Introduction**

Every day, the growing use of herbal remedies is moving closer to setting new records, and the practise of "herbalism" is offering the best possible outcome for the medical care sector. One of the most crucial parts of herbalism is the pharmacological testing of herbs [1]. This testing provides information about potential safety risks and guarantees that herbal remedies have desired features that permit their use in clinical investigations. It is often known that a large number of people nowadays prefer herbal medications to conventional ones; this preference may be explained by the fact that herbal medications provide fewer health risks [2]. This effort is a component of the effort to provide enough scientific evidence to support the herb's unrealized potential, as revealed in earlier publications. One of the unstudied plants whose potential medical benefits is still awaiting a scientific method is *Zizyphus xylopyrus*. [3, 4] Based on pertinent published studies, multiple screening models were built to examine the antiulcer activity of the plant isolate fraction.

## **Overview of phytochemistry**

When we use the term "phytochemistry" in its most literal meaning, we mean the study of phytochemicals [5]. These are substances that come from different kinds of plants. More narrowly, the terms are widely employed to describe the great majority of secondary metabolic products that are potentially found in plants [6]. It is common knowledge that several of these provide protection from plant-damaging insects and illnesses. They also provide protection and carry out a variety of tasks that are advantageous to human clients.

## **Improvements in phytochemical analysis methods**

The continuous advancement of spectroscopic and chromatographic techniques for analysis has simplified the process of identifying and isolating biologically active compounds [7, 8]. Rapid communication on all aspects of natural product research is encouraged by *Phytochemistry Letters*, including structural clarification, biotechnology, pharmacology, ethnobotany and traditional use, genetics, analytical assessment of herbal remedies, Chemical ecology, metabolomics, natural product metabolism, and biosynthesis of natural products [9].

The Phytochemistry Unit has skills in the following areas, and it plays a very essential part in the process of collecting plants that are of interest to HMRC. These areas are as follows:

Gathering and preparing plant sample

Sample preparation for use in a bioassay involving plants. Evaluation of Phytochemical Potential.

Methods like LC-MS analysis, Flash Chromatography, Preparative TLC, Preparative HPLC, GC-MS, HPLC-UV, and HPLC-Diode Array were used to partition and separate bioactive chemicals.

Preparation and analysis of standardised extracts using high-performance liquid chromatography (HPLC).

## **Medicinal plants' antimicrobial properties [10,11]**

Many scientists have investigated the antibacterial qualities of plants worldwide, but especially in Latin America. The effectiveness of 122 distinct species of medicinal plants was investigated in an Argentinean study [12]. A total of twelve compounds that were extracted from these plants were found to be effective in inhibiting the growth of *Staphylococcus aureus*, ten compounds that prevented the growth of *Escherichia coli*, and four compounds that inhibited the development of *Aspergillus niger*. The most potent ingredient was taken out of *Tabebuia impetiginosa*. Compounds isolated from *Parthenium argentatum* have demonstrated antibacterial action against a variety of bacteria and fungi, including *Torulopsis*, *Hansenula*, *Klebsiella pneumoniae*, *Candida albicans*, and *Pseudomonas aeruginosa*. [13].

When the compounds isolated from nine different plants in Uruguay were tested for their antimicrobial properties, they exhibited antibacterial action against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*, but not against *Candida albicans* or *Saccharomyces cerevisiae*. *Vatairea macrocarpa* was discovered to suppress the growth of *Klebsiella* spp. and *S. aureus*, whereas extracts of *Eucalyptus* species were found to reduce the growth of soil fungus. In a more thorough study, the antibacterial qualities of extracts from 120 plant species from 28 families

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were examined[14]. It was demonstrated that five extracts from four distinct plants could inhibit *P. aeruginosa* growth, and that 81 extracts from 58 different plants could effectively inhibit *S. aureus* development. In another study, it was demonstrated that essential oils isolated from *Croton triangularis* leaves had antibacterial and antifungal (*C. albicans*) action. It has been established that the extracts of *Xylopia sericea* and *Lippia gracilis* contain antifungal qualities. Extractions from 30 plant species were tested for their antibacterial activity and cell toxicity against 5 bacterial and 2 fungal species [15]. It was shown that only one species of *Combretum duarteianum* had any antibacterial effect at all, and that 70% of the plant ethanol extracts were harmful to cells. The antibacterial and toxicity of extracts from *Arthemis sativa* have been investigated. The "thin leaf guaco" plant, *Mikania triangularis*, was found to have antibacterial activity against *Pseudomonas aeruginosa*, *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* under a microscope. Subsequently, the study investigated the bactericidal effectiveness of anacardic acid and totarol against methicillin-resistant *Staphylococcus aureus* (MRSA) as well as their potential synergistic relationship with methicillin. Research on the utilisation of plants as therapeutic agents should therefore be given top priority, especially when it comes to treating bacteria that are resistant to antibiotics[16]. The purpose of this study was to investigate the potential efficacy of plant extracts and phytochemicals against common microorganisms as well as hospital-identified MDR strains of bacteria. We also investigated the potential for extracts possessing antibacterial activity to collaborate with antibiotics in the fight against drug-resistant microbes[17].

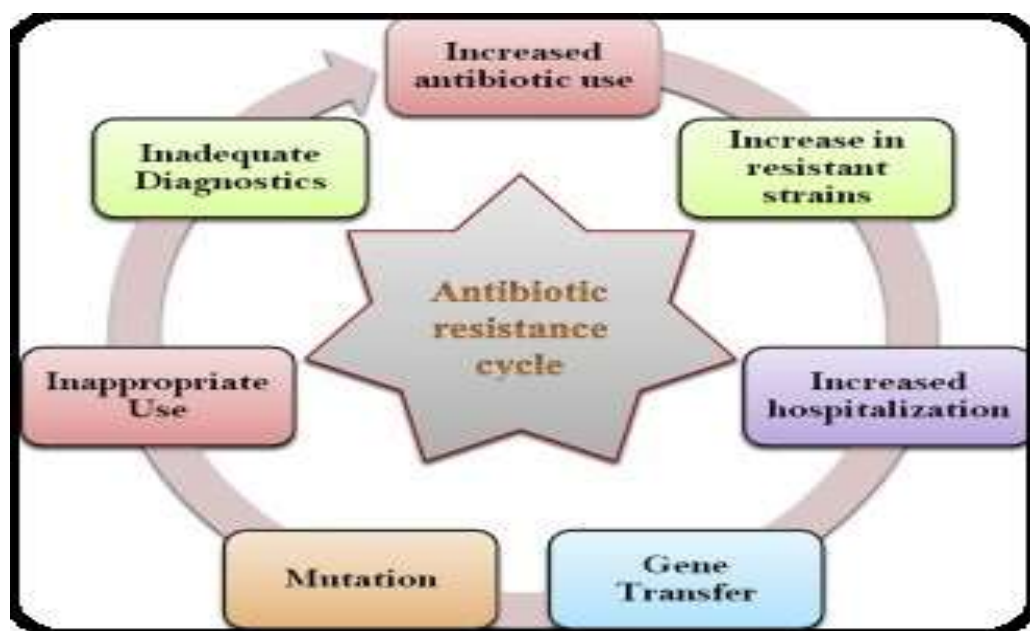


Fig.2 Various mechanism of antibiotics bioactive compound

Table 1 List of Important medicinal herbs showing antimicrobial activity[18]

| Botanical name               | Family           | Parts used |
|------------------------------|------------------|------------|
| Saussurea lappa              | Asteraceae       | RT         |
| Xanthium sibiricum           | Asteraceae       | FT         |
| Hippophae rhamnoides L.      | Elaeagnaceae     | LF         |
| Abrus cantoniensis Bge.      | Fabaceae         | AP         |
| Astragalus membranaceus Bge. | Fabaceae         | RT         |
| Bupleurum chinense DC.       | Apiaceae         | AP         |
| Foeniculum vulgare Mill.     | Apiaceae         | FT         |
| Ligusticum chuanxiong Hort.  | Apiaceae         | RT         |
| Aristolochia debilis         | Aristolochiaceae | RT         |
| Aristolochia mallissima      | Aristolochiaceae | LF         |
| Trigonella foenum-graecum L. | Fabaceae         | SD         |
| Lindera strychnifolia Vill.  | Lauraceae        | RT         |

| Botanical name               | Family    | Parts used |
|------------------------------|-----------|------------|
| Mentha haplocalyx Briq.      | Lamiaceae | AP         |
| Scutellaria barbata D.Don    | Lamiaceae | WP         |
| Fritillaria thunbergii Miq.  | Liliaceae | ST         |
| Cassia obtusifolia Vahl.     | Fabaceae  | LF         |
| Glycyrrhiza uralensis Fisch. | Fabaceae  | RH         |
| Sophora flavescens Ait       | Fabaceae  | RT         |

**AP**, aerial part; **BK**, bark; **FL**, flower; **FT**, fruit; **FS**, fruit shell; **LF**, leaf; **RH**, rhizome; **RT**, root; **SD**, seed; **SP**, spike; **ST**, stem; **WP**, whole plant.

### **Describe the profile of Zizyphus Xylopyrus**

Within the Malvaceae family of broadleaf evergreens, Zizyphus is a vast genus including over 150 species. The genus, which is found in tropical and subtropical regions on all continents, comprises annuals, perennials, shrubs, and small trees that grow to a height of 1 to 10 m. In Pakistan, it is represented by eighteen intraspecific and specific taxa[19]. The leaves are palmately lobed (with three to seven lobes) or alternating, unlobed. With five petals, generally red, pink, orange, yellow, or white, the blossoms are striking.



Fig.3 Description of leaf part of Zizyphus Xylopyrus

### **BiologicalSource:**

The entire dried herb Zizyphus Xylopyrus (Retz) is used in the medication. (Family:Rhamnaceae)

### **Geographical Source**

The plant is widespread in Bangladesh, India, Nepal, Sri Lanka, and Assam.

### **Large-scale Features of the Zizyphus Xylopyrus species [20]**

It is a highly branching, one to two metre (three to six foot) annual herb or perennial sub shrub, with younger portions coated in a whitish grey down. Stem is circular, frequently tinged with purple hue. The simple leaves are alternating, rounded to oblong, the border irregularly crenate or serrated. Like hibiscus flowers, the auxiliary blooms are solitary or in terminal groups, with five yellow petals and numerous stamens joined in a tube. There are ten to fifteen carpels. Calyxes have a split centre that is lobed. Three to five kidney-shaped, dark brown or black seeds with little stillate hairs make up 36 seeds. Fruits are flattened, papery, and organised like a wheel's spokes.

### **Chemical composition of Zizyphus Xylopyrus [21]**

Zizyphus Xylopyrus lacks tannins but does contain gum resin and mucilage. Two sesquiterpene lactones, designated as alantolactone and isoalantolactone as well as gallic acid, were extracted from the plant using petroleum ether. Zizyphus Xylopyrus leaves, roots, and stems are used in traditional



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medicine to cure rheumatism and as demulcents, emollients, and diuretics because they contain a significant amount of mucilage[22]. A few of them are recommended as cooling medications for fevers. The some of the medical applications for various Zizyphus Xylopyrus components.

Blossoms:-are applied locally on ulcers and blisters.

Leaves: There have been reports of tocopherols and  $\beta$ -sitosterol present in its leaves. The leaves, according to local medical professionals, are very helpful in managing diabetes mellitus. Leaf decoction is applied topically to areas that hurt. Additionally, leaves are utilised as a treatment for liver problems.

Barks: They are used to treat fever, haemorrhage, pulmonary sedation, diuretics, and anthelmintics. The bark reduces sweating and quells thirst by eliminating "vata."

Root: Zizyphus Xylopyrus lacks tannins but has gum resin and mucilage. Two sesquiterpene lactones, designated as alantolactone and isoalantolactone as well as gallic acid, were extracted from the plant using petroleum ether. Root extracts that were extracted using various solvents demonstrated antifungal and antibacterial properties. The root is said to be tonic, astringent, and cooling. In leprosy, hemorrhagic disease, and stroke, it is regarded favourably.

Seeds: Raffinose is the sugar component found in its seeds. Its seed oil has been chemically analysed, and the results show that it contains 12,13 epoxyoleic acid, malvalic, sterculic, oleic, palmitic, and stearic acids.

## **2. Material and Methods[23]**

### **Plant material and chemical**

Zizyphus xylopyrus leaves were collected between March–June 2022 from the college campus in Bhopal, M.P., India. The plant (Leaves) was validated by the Department of Botany, Dr. H. S. Gour Vishwavidyalaya, Sagar (M.P.) by (Herbarium no. Bot/413). Analytical grade chemical was utilised throughout.

### **Sample Setting Up**

The leaves of Zizyphus xylopyrus were carefully cleaned using double-distilled water after rinsing with tap water, and then allowed to dry in the shade for a period of seven days. Using a household mixer grinder, the dried plant was ground into a fine powder. For upcoming research, the plant powder was kept in desiccators.

### **Extraction of flavonoids**

Z. xylopyrus leaves weighing about 100 grams were shade-dried at room temperature. The plant material that had been shade-dried was roughly ground up and then extracted using petroleum ether in a soxhlet device. Until the material had undergone deffatting, the extraction process was maintained. The marc that was produced after ethanol was extracted from petroleum ether using a Soxhlet system. To accomplish full extraction, the extraction was carried out over the course of six to seven days. After being concentrated, the extract was dried to a set weight. Physical characteristics of the drug extracts, including consistency, colour, odour, and taste, were assessed. the existence of the desired phytochemicals, such as saponins, tannins, and flavonoids.

### **Pharmacological Evaluation**

#### **Zizyphus Xylopyrus contain antimicrobial studies[24]**

Gram positive and gram negative bacteria as well as fungi were the targets of the evaluation of amoxicillin and its plant extract showing in vitro antibacterial activities. The inhibition zone surrounding the tested material was evaluated to determine the antibacterial activity of each extract. In comparison to the amoxicillin, all plant extract exhibit an enhanced zone of inhibition against investigated bacteria and fungi. Ethyl acetate was active against gram positive (staphylococci) and gram negative (E. Coli and pseudomonas aeruginosa) bacteria, whereas ethanolic extract had reduced activity. Except aqueous exhibit fatal antifungal action towards Candida Albicans, whilst n-hexane were found to be active against aspergillus flavus. Comparing the standard drug antibacterial and antifungal properties, it was found that n-hexane produced encouraging outcomes at the 10 ppm

concentration level. It was possible to see that the former had demonstrated encouraging outcomes. Tweedy's Chelation theory provides an explanation for the metal complexes' higher inhibitory activity.

### Bactericidal action

#### Finding the percentage of inhibition

Extracts of *Z. Xylopyrus* were tested for their antibacterial efficacy against a variety of bacteria, including *Salmonella typhi*, *Bacillus pumalis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, and *Enterobacter aerogenes*. The test organism was grown in nutrient broth for 18 hours before being transferred to sterile nutrient agar plates. After waiting 30 minutes, a sterile 6 mm borer was used to drill holes in plates. Dimethyl sulfoxide (DMSO, less than 1%) stock solutions (3mg/ml) were used to prepare the test samples. The crude methanolic extract and fractions were applied to the wells at a concentration of 100 l. As a positive control, amoxicillin was used, and as a negative control, DMSO concentrations of less than 1% were employed. The zone of inhibition was calculated (in millimeters) in comparison to the positive control using the following formula.

$$\% \text{ Inhibition} = \frac{\text{Zone of Inhibition of Sample}}{\text{Zone of Inhibition of Standard}} \times 100$$

**Table 2** Crude methanolic extract and different fractions of *Z. Xylopyrus* have antibacterial properties.

| Table 2<br>Antibacterial<br>activity of<br>crude<br>methanolic<br>extract &<br>various<br>fractions of<br><i>Z.Xylopyrus</i><br><br>Name of<br>Bacteria | Zone of<br>Inhibition of<br>standard<br>(Amoxicillin)<br>10µg/Disc | Crude<br>Extract<br>Methanol  |                   | <i>n</i> -hexane              |                   | EtOAc                         |                   | Aqueous                       |                   |
|---------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------|-------------------------------|-------------------|-------------------------------|-------------------|-------------------------------|-------------------|-------------------------------|-------------------|
|                                                                                                                                                         |                                                                    | Zone of<br>Inhibition<br>(mm) | Inhibition<br>(%) | Zone of<br>Inhibition<br>(mm) | Inhibition<br>(%) | Zone of<br>Inhibition<br>(mm) | Inhibition<br>(%) | Zone of<br>Inhibition<br>(mm) | Inhibition<br>(%) |
| <i>S.epidermidis</i>                                                                                                                                    | 25                                                                 | 8                             | 36.61             | 16                            | 43.84             | 15                            | 55.38             | 11                            | 48.46             |
| <i>S.typhi</i>                                                                                                                                          | 28                                                                 | 9                             | 34.03             | 17                            | 53.65             | 15                            | 60.86             | 17                            | 65.55             |
| <i>S.aureus</i>                                                                                                                                         | 24                                                                 | 9                             | 35.66             | 11                            | 40.46             | 9                             | 40.76             | 8                             | 44.61             |
| <i>P.aeruginosa</i>                                                                                                                                     | 28                                                                 | 16                            | 65.55             | 12                            | 47.03             | 19                            | 68.96             | 19                            | 70.66             |
| <i>B.pumalis</i>                                                                                                                                        | 25                                                                 | 13                            | 52.00             | 15                            | 60.00             | 18                            | 72.00             | 10                            | 40.00             |
| <i>E.aerogens</i>                                                                                                                                       | 28                                                                 | 14                            | 62.00             | 17                            | 66.00             | 17                            | 75.00             | 11                            | 50.00             |
| <i>E.coli</i>                                                                                                                                           | 28                                                                 | 0                             | 0                 | 9                             | 30.62             | 14                            | 46.14             | 10                            | 45.74             |

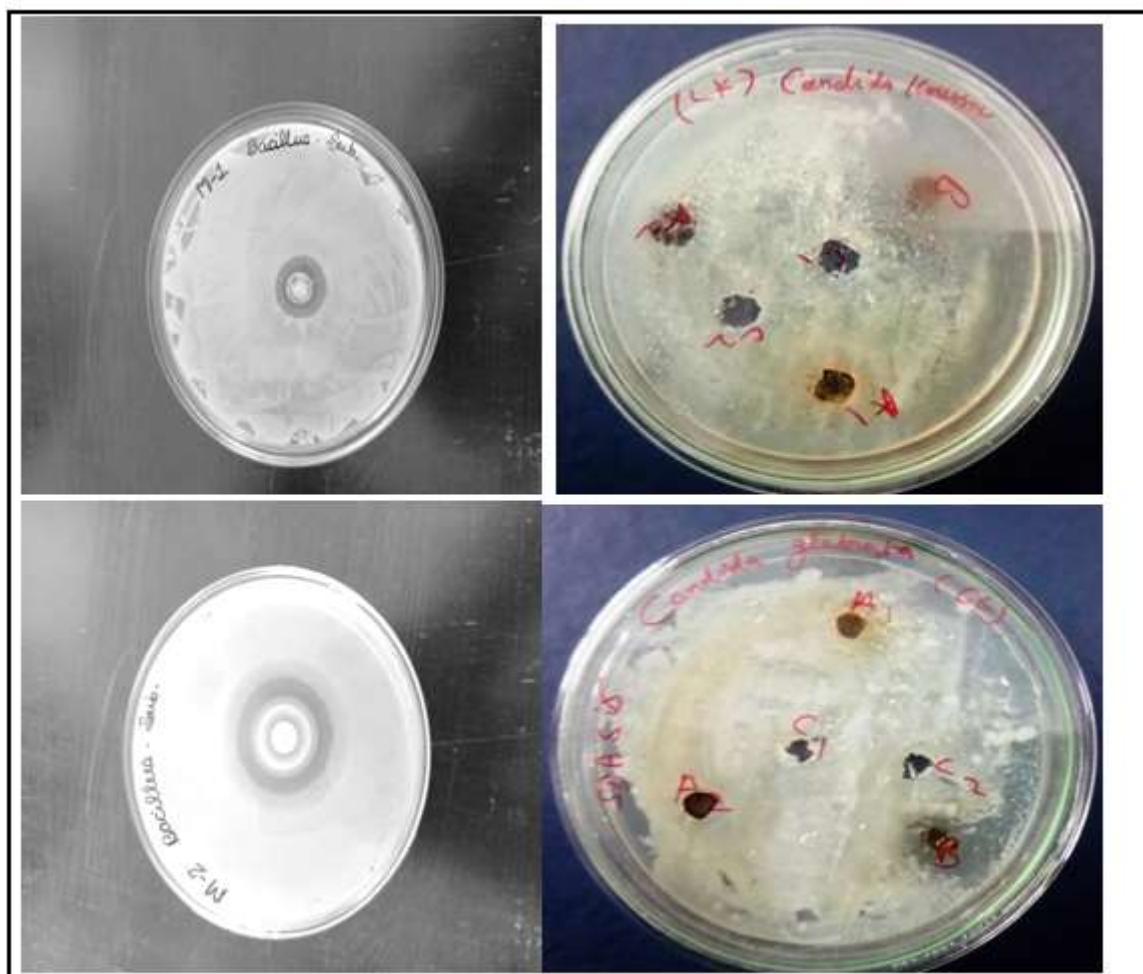


Fig.4 Systematic diagram representing the antimicrobial activity in various extracts

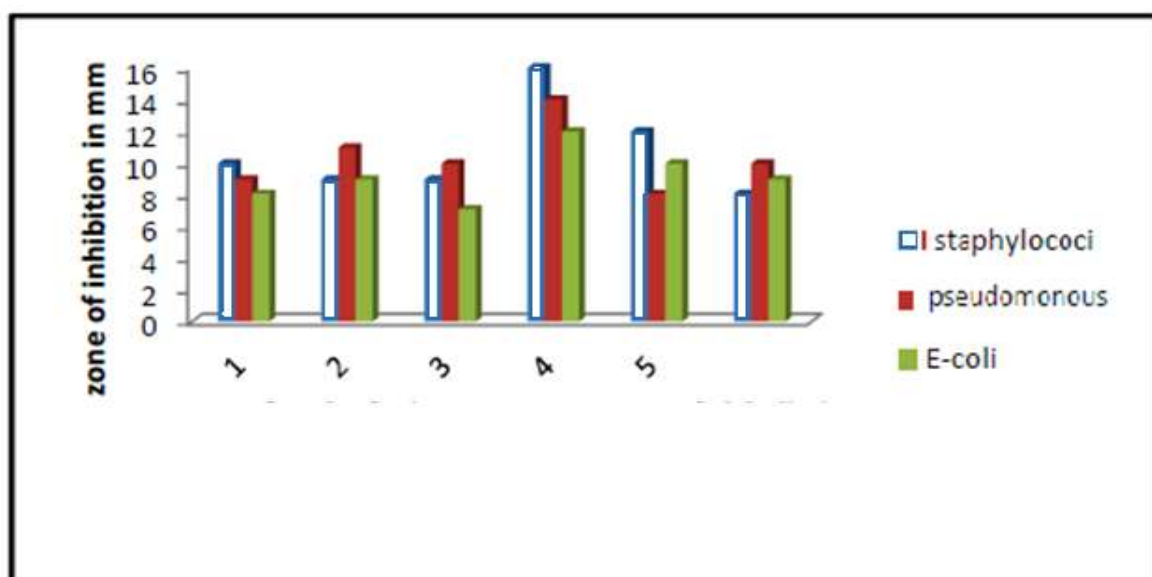


Fig.5 Bar graph of antibacterial activity of Z. Xylopyrus

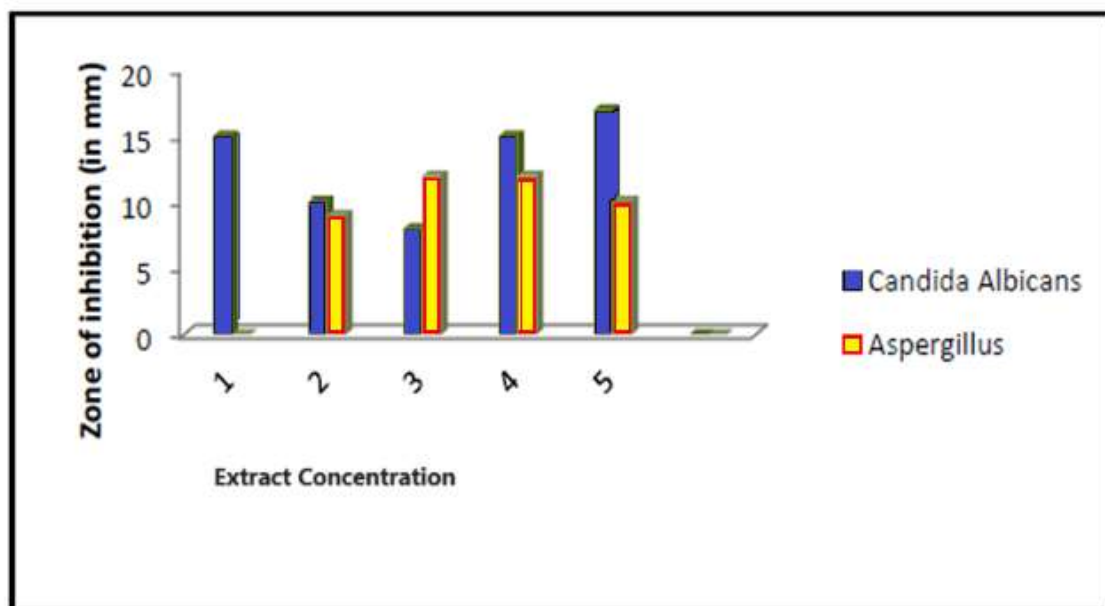


Fig.6 Bar graph showing antifungal activity of *Z. Xylopyrus*

**The minimal inhibitory concentration (MIC) is determined.**

The test samples' MIC<sub>50</sub> against the test organisms at dosages of 0.9, 1.5, 2.1, 2.7, and 3.2 mg/ml were evaluated following the computation of the % inhibition. Samples and organisms were placed into test tubes with 4 millilitres of sterile nutritive broth; the tubes were then cultivated at 37 °C for a whole day. After a 24-hour period, data were recorded using the percent clarity in comparison to the negative control group. The nutrient-rich broth medium functioned as a negative control.

### 3. Result and Discussion

There is no denying this plant's therapeutic potential. This study opens the door for more research on the phytochemical and pharmacological characteristics of the plant. Numerous beneficial chemicals are present in this plant, according to phytochemical research. For example, the ethanolic extract, ethyl acetate, and isolation of *Z. xylopyrus* leaf contain phenols, flavonoids, saponins, and tannins. Rutin and Quercetin are the main flavonoids in this plant, based on TLC and co-TLC profiles. Research from the past has indicated that this plant contains a lot of flavonoids. Tests were conducted to determine the antibacterial activity of *Z. Xylopyrus* crude extracts against a range of bacteria, including *Salmonella typhi*, *Bacillus pumalis*, *Pseudomonas aeruginosa*, *Salmonella aureus*, *Streptococcus pneumoniae*, and *Enterobacter aerogenes*. A culture of the test organism cultured in nutrient broth for eighteen hours was plated on sterile nutrient agar plates to create bacterial lawn. Wells were bored into the plates using a sterile 6 mm borer after 30 minutes. The test samples were diluted in sterile dimethyl sulfoxide (DMSO, less than 1%) to a stock solution of 3 mg/ml. 100 l of a crude methanolic extract or fraction were applied to each well. A positive control was the antibiotic amoxicillin, whereas a negative control was DMSO concentrations less than 1%. The advent of novel illnesses, such as those caused by *Enterococcus* and *Staphylococcus* species, agents of many intra-hospital infections, and antibiotic resistance to current medications have drawn increased interest to research on medicinal plants during the past few decades. For instance, *S. aureus* used to be vulnerable to various medications, but now it is resistant to them. It is currently resistant to a number of antibiotics, including tetracyclines, penicillin G, gentamicin, macrolides, and lincosamides. *Z. xylopyrus*, which is used locally as a convulsant, analgesic, and tranquillizer and is prescribed for the treatment of anxiety and insomnia, satisfies our requirements for investigating novel bioactive chemicals originating from plants.



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